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IMMUNOLOGICAL STUDY OF PARASITES

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What particularly attracts us in the recent trends in parasitology ~~is a series of~~ studies concerning parasite immunity. Among them, studies on parasite immunity (infection resistance) in particular are deeply interesting.

On parasite immunity, in the past infection immunity was observed for malaria, and the cure for protozoan Leishmania tropica ~~were~~ known. However, ~~to obtain~~ curative immunity to the so-called coarse parasites (vermes), ~~the subject of this article~~ was considered impossible. Recently a curative immunity was found for certain kinds of domestic animals parasites by innoculating infected larvae whose activity was weakened by X-ray. This proof that a curative immunity can be guaranteed in parasitic diseases by vaccination is attracting attention. Also, in the immunological diagnosis of parasites remarkable progress has been made, which has been introduced into clinical aspects and immunological studies with much success. ~~In the following~~, studies on the immunological diagnosis of parasites and on parasite immunity ~~will be~~ described.

I. The Immunological Diagnosis of Parasites

1. Immuno-sero Diagnosis

The diagnosis of parasitic diseases is carried out by proving the existence of eggs, larvae from such excrements as feces, urine, expectoration or from blood and tissues. But even if parasites exist, they are not always detected, for instance, when parasites are too weak or aged to oviposit,

when there are unisexual male vermen parasites or when parasiting occurs in abnormal places. Also parasites are affected by treatment and temporarily suspend their function of ovipositing. In such cases an indirect proof, such as immuno-sero diagnosis is necessary.

When the parasitic infection in a certain area is to be ascertained, or when success in the prevention of parasitic infection is to be obtained, it is not easy to carry out collective stool tests and collective blood tests with limited personnel and within a limited time. In such cases a simplified method would be very convenient. Therefore, immuno-sero diagnosis may be used as a precision test to ascertain clinical diagnosis with more certainty, as a yardstick for epidemiogical determination, or as a screening method. The following intradermal test falls into the latter category.

Intradermal Test

Interdermal tests observed in parasitic diseases are all immediate types. Because this test has comparatively special characteristics and is moreover carried out in an extremely simplified and convenient manner, its value is great if it is used properly. In the following the interdermal test for lung flukes generally used in Japan will be described.

a) Interdermal Test of Lung Fluke Disease

Because the interdermal test for lung fluke disease is extremely simple and is not hazardous, the test is used in collective tests to screen lung flukes in epidemic areas. On the other hand, because it is idiosyncratic, it is also used to differentiate other chest diseases such as tuberculosis. In this case, the interdermal test positive is more reliable than the interdermal test negative. In other words, when this test is negative, lung flukes can be excluded.

If the intradermal test is positive, a further precise test is necessary. For, once the interdermal test is positive the positive reaction is almost permanent even after complete cure. However, the test positive does not necessarily mean that the person is infected with this disease.

b) Interdermal Test Formula and Criteria for Determination

1) Antigen: The interdermal test antigen for lung flukes is called V.B.S. lung fluke extract antigen. The

National Institute of Health supplies this for general demand.

2) Formula: The extract antigen is injected in the bend of the left antegrachium of the patient so that about 3-4 mm swelling will result, and the longest and shortest diameters of the swelling immediately after injection are measured. (The volume of injection at this time is about 0.005-0.01 cc). In 15 minutes the longest and shortest diameters of the swelling are measured again. The mean values of the longest and shortest diameters immediately after and in 15 minutes of injection are calculated respectively. Subtracting the average diameter immediately after injection from the average diameter 15 minutes later gives the swelling difference. Over 5mm difference is considered test positive, while 4mm is considered pseudo-positive; and less than 3mm test-negative.

In the case of test positive, swelling like an insect bite begins several minutes after injection, sometimes producing "artificial leg-like shapes." The swelling reaches the maximum in 10-15 minutes, and gradually diminishes. In 24 hours it disappears completely.

In collective intradermal tests in epidemic areas of lung flukes, 20-40% of the examinees with test positive are found to be parasite egg positive. By age brackets, the rate of parasite egg positive is high in the 10-20 year bracket, and drops as the age progresses. This seems to be because the disease is cured among the persons of advanced ages.

There is comparatively small group reaction to other kinds of parasitic diseases. Only some persons with clonorchis sinensis show test positive.

c) Intradermal Test for Schistosomiasis

In addition to lung flukes, for Schistosomiasis, as a paratistic disease the intradermal test is widely used. WHO has also taken the intradermal test for Schistosomiasis and examined its antigen, formula, and criteria for determination. There are no marked differences from lung flukes. There are three kinds of Schistosomiasis, Manson's disease, Bilharzian disease, and Schistosomiasis japonicum. However, they cannot be differentiated by intradermal tests. This intradermal test positive sometimes appears also in those with cercaria dermatitis and is caused by the cercaria of schistosome in animals.

d) Intradermal Tests for Other Parasites

Intradermal tests serve as important clues in detecting the infection of echinococcosis, even though this is rarely found in Japan, because it is difficult to directly prove the existence of vermes.

Intradermal tests are also attempted for filaria. In this case, antigen is manufactured from the adult of Diroilaria immitis. Accordingly, those who have been infiltrated by the filaria of animals will show test positive. For instance, the disease known as Tropical Eosinophilia is sometimes complicated by a light lung disease apex. The cause of this disease was unknown, but it is now suspected that the cause is infection of animal filaria such as canine filaria. In this case the intradermal test of filaria is reported to be positive. The test is widely used also for trichinosis.

Although there are considerable records of intradermal tests being applied to hookworm diseases and ascariasis, the majority of the group of examinees will show test positive, because those with past infections of these diseases will almost all show test positive, even though the life of these vermes in the human body is short. Therefore, this test will be of almost no value in screening.

In order to widely apply intradermal test, it is necessary to constantly prepare antigens of various parasites. In Japan only the lung fluke antigen is being manufactured.

Complement Fixation Test

The complement fixation test, different from the intradermal test, changes from positive to negative with the death of parasites. It is an indispensable diagnostic method but its idiosyncracy is not as strong as the intradermal method. The complement fixation test appears positive in 85-90% of the examinees with lung fluke egg positive. Even healthy examinees show test positive, but in this case it is characteristic for the antibody value to be markedly low.

The record of complement fixation tests for those who are infected with lung flukes shows that the antibody value of the complement fixation test tends to drop rapidly immediately after cure, and many of them show negative in half a year. In the instances which show no decrease in the antibody value many show recurrence in two or three months, even if the parasite eggs are not detected.

The [illegible] in addition to [illegible] for filariasis, schistosomiasis, and [illegible]. Although it is reported that the complement fixation test in schistosomiasis shows a positive reaction in the comparatively early stages of infection, the experiment by the author indicated that the time of change over to a positive reaction in the case of lung flukes roughly coincides with the time the maturation of vermes. In the same experiment, the complement, the complement fixation test did not show positive in the larval period infection. Also in the case of the unisexual parasiting of schistosoma japonicum, either male or female, it rarely grows to adulthood. In this case, the positive reaction of the complement fixation test could not be proven through theexperiment of the author.

Precipitation Test

The precipitation test for parasitic diseases rarely shows remarkable results, consequently, its practical value is small. Therefore, various methods have been devised. In other words, in the case of schistrosoma, trichinosis, and lung flukes, antigens are adsorbed by the particles of Bentonite or Cholesterol for an agglutination reaction (rapid flocculation test).

2. Immuno-biological Diagnosis

This method uses parasite larvae of a certain period as antigens and diagnosis is made by observing the changes appearing in the larvae.

Sarles Phenomena

Sarles (1938) first reported that when the Nippostrongylus muris larva was placed in the serum of the infected animal, the formation of sediment around the body of the larva, mainly around the mouth and the excretory, was observed. Thereafter such phenomena was proved in Trichinella spiralis, canine hookworm, American hookworm, and trichostrongylus orientalis and other nematodes. And, when the micro-filaria found in the blood of a filaria patient is placed in the serum of the filaria patient, the phenomenon of the filaria agglomeration has been proved.

The foregoing have been reactions observed in nematodes, but various other kinds of reactions have also been proved for flukes with more complicated growth rings.

Miracidial Immobilization Test (MIT)

Miracidium in the egg of schistosoma is used in this test. This test was first developed by Senterfit (1953). He found, when actively moving miracidium are placed in the serum of an animal infected with schistosoma mansoni, it immediately stopped its movement. In this case non-activated serum is used, and it is reported that such a phenomenon is not observable in normal serum. It is believed that the cessation of miracidium movement is due to the formation of sediment around the cilia of the body and the subsequent cessation of the movement of the cilia. But, it is reported that in the test made on schistosoma mansoni patients 22 examinees (51%) out of 43 showed test positive.

Circumoval Precipitin Test (COP-test)

Oliver-Gonzalez (1954) discovered that when the egg of schistosoma mansoni was placed in the serum of an animal infected with schistosoma mansoni, a spheral or chain-like formation was observed around the ovitesta of the egg. According to him, this formation is the combination of the antigen in the egg filtered through the ovitesta, and then combined with the antigen in the serum. He further claims that even from the results of the adsorption test, this is the true antigen-antibody reaction. The test in accordance with this method made by the author on patients infected with schistosoma japonicum showed that out of 32 examinees, 31 examinees (96.8%) showed a positive reaction. This completely agreed with the results of the complement fixation test carried out at the same time.

According to Oliver-Gonzalez, this test will change to negative in six months after the complete cure. While it is reported that the test would appear positive in the case of unisexual parasiting, this could not be proved by the author's experiment.

In any case, this test has such advantages as the operation of the test being simple, the reaction being extremely clear, and the test egg can be used for one month if it is placed in an N/15 NaCl solution after separation from liver at 5°C.

Cercarien Huellen reaction (CHR-test)

This test was first reported by Vogel (1949). According to him, when the cercaria of schistosoma is placed in the

serum of the host, changes similar to ecdysis are observable. That is, the surface of the cercaria is covered with two-layer cuticles, and when these are peeled off, and the outer-layer cuticle is stretched to form wrinkles, this gives the appearance of being covered with a sheath. This change does not occur in inactivated serum at 56°C. Vogel reports that this reaction is observable in the comparatively early period after infection, and also in the case of unisexual parasiting. However, the existence of matter that causes a reaction that is sometimes confused with this has been proven. Therefore, care should be exercised in determining the results of this test.

Cerdarial Agglutination Test (CA-test)

This test and the above CHR-test are often confused. This latter test occurs in serum inactivated at 56°C, thus essentially differing from the former. When this test is positive, it is reported that cercaria assemble to form masses which seem to be caused by the formation of sticky sediments on the surface of the cercaria (Liu and Bang, 1950). However, there have been no differentiating characteristics. According to Oliver-Gonzalez (1955), this test showed positive in all patients soon after infection, but in the case of chronic patients with a comparatively long period of infection, only four (6.1%) out of 49 cases showed positive reactions. Therefore, many aspects of this test should be clarified.

Fluorescent Antibody Test (FA-test)

The method to prove the existence of antibodies by the so-called FA-test in the realm of parasites was already attempted for Trichinella spirals, Nippostrongylus muris (Jackson, 1959, 1960) and flukes (Lewart, 1958). However, diagnosis of flukes.

When the cercaria or midacidium of flukes is fixed in 10% formaline water, soaked immediately in the serum of the infected animal, then soaked again after rinsing in flourescin tagged globulin, and then observed with a flourescent microscope, the cercaria or miracidium emit flourescent light. The fluorescent light cannot be observed in normal serum.

Originally, fresh cercaria had to be fixed each time by formalin, but this was improved afterwards. Thus, when rhodamine or albumin of cattle is added to 10% formalin

water, it is reported that [illegible] endures use for a comparatively long period. Also the volume of serum needed for this is very small. Then, one drop of blood obtained by piercing the finger tip can be adsorbed by filter paper and then taken back to the laboratory for extraction by salt water and used in place of serum. This aspect is convenient for collective tests.

II. Studies on the Immunity (Infection Resistence) of Parasites

As far as the immunity of parasites is concerned, that infection can be prevented by so-called antigen immunity, that is by vaccination, has not been proved yet. But, in the protozoan diseases, for instance, malaria, infection immunity, that is, in the period of chronic infection of malaria, shows a remarkable increase of resistence to re-infection. The Leishmania tropical found in the countries along the Mediterranean coast is a protozoa that forms ulcerative phyma on skin. It is known that once man is infected with this and is completely cured, he gains complete immunity. It is reported that among the inhabitants of epidemic areas of this disease, a custom was developed that juice flowing from the abscess of the skin of the infected patient was innoculated into un-infected infants in order to avoid the formation of ugly scars.

As for parasites (vermes), the subject of this article, many facts that seemed to support the possibility of a certain degree of infection immunity against hookworm disease and other parasi[illegible] have been reported. Also on the subject of immunity a[illegible] the cure, so-called cure immunity, after the cure, so-called cure immunity, various studies on various parasites have been made. Dr. Fujinami Kagami of Japan (1916) is believed to have been the first to have noted this fact. Dr. Fujinami, while studying schistosoma japonica, noted that while many head of cattle in the epidemic areas would die from schistosoma infection, horses showed almost no such diseases. He therefore conducted the following experiment.

When he infected horses raised in the epidemic area and horses recently purchased from non-epidemic areas with schistosoma japonica at the same time, over 10,000 vermes were found in the latter horse in the contact test, while not a single vermes was recognized in the already infected horse. Also observation of microscopic specimen taken from various parts of the viscera of both types of horses agreed with this result. Thus, Dr. Fujinami reported that "obtaining

immunity from [illegible] from the [illegible] organism diseases, has not been given too much attention by scholars. However, in view of the above experience, in diseases caused by certain parasitic animals, it is possible that certain host bodies gain the ability to hinder the growth of parasites in their own body and so, to resist re-infection under certain conditions." Dr. Fujinami added that obtaining immunity will differ according to the kind of animal, and so no generalization could be made. Thereafter many facts to support the theory of Dr. Fujinami have been discovered with such parasites as Nippostrongylus muris, canine hookworm, Trichinella spiralis. However, in all cases the immunity was relative, and no perfect immunity was obtained.

Accordingly many regard obtaining perfect immunity as impossible. And, even if obtaining immunity is possible, the method of antigen immunity would be unthinkable. Instead it would be necessary to be infected with the parasite concerned, that is to go through the infection of the completely grown adult parasite. Thus, obtaining immunity by the larva infection was regarded as impossible also. Further, even if immunity was to be obtained after infection, infection with a volume close to a fatal dosage was necessary. Therefore, the damage to be suffered by the host prevented the practical use of this method.

Recently however Scot veterinarians led by Jarrett (1959) clarified that when the infected larvae of parasites od domesticated animals were used, after hindering part of their activity through X-rays, the infected animals obtained complete immunity without any notable side-effects. As a result, research in this field began once again. Dr. Stoll, Director of Parasitology of the Rockefeller Institute of America made a speech in 1960 entitled, "The Worms; Can We Vaccinate Against Them?" On that occasion he introduced the accomplishments of Jarrett et al and reportedly stated his hopeful view that parasite infection, other than protozoa, would be possible in the near future.

The prevention or eradication of parasites in Africa and other tropical zones, unlike Japan, cannot be achieved by collective vermicides or partial interruption of the parasite life cycle. Therefore, it is the prevailing view among European and American parasitologists that in addition to these, infection prevention by vaccination is a strong weapon against parasites. In view of this, studies in this phase will increase more and more. Yet, even an effective vaccine against parasites cannot be immediately used on human bodies, unlike animals. Also in view of the present lack of

a method to determine the degree of [illegible] animals does not immedi[illegible] in human bodies. But, this proble[illegible] the following the accomplishments of Jarrett, et al and other affiliated achievements will be discussed.

On the Acquisition of Immunity to Distiocaulus viviparus by Cattle.

This is a nem[illegible]de that parasites on the lung of cattle. The adult is thread-like and 5-8-cm in length. Cattle infected with this vermes develop difficulty in breathing, a loss of appetite, diarrhea and fever, often pneumonia, and die. In England this epidemic occurred explosively on livestock farms where many cattle would die at once. Yet, those which escaped death were believed to be un-infected in subsequent epidemic periods.

In order to ascertain this fact, Jarrett (1959-1960), et al experimentally infected many calves with various amounts. As a result of this it was discovered that a nearly fatal dose was necessary to obtain complete immunity. Thereupon, such methods of giving immunity as injecting the extracted solution from adult vermes or burying dead vermis in subcutis were attempted. Consequently, the role of antibodies in blood was observed, but the prevention of vermis infection was impossible. Thereupon, larvae irradiated with 40,000γ of X-ray was used for immunity and cattle obtained immunity without any side-effects in the subsequent infection.

Jarrett, et al gave 1,000-4,000 larvae irradiated with 40,000γ X-rays to groups of 10 head of cattle once or twice; 10,000 units of normally infected larvae were used for infection. The results are shown in Table 1.

Table 1

Experiment on Giving Lung Vermes Immunity to Cattle

①実験番号	②第1回接種 X線(40,000γ)照射 仔虫数		③第2回接種 X線(40,000γ)照射 仔虫数		④感染 正常仔虫数		⑤剖見 肺における成虫生体数
1.	1,000	← 42日 →	4,000	← 42日 →	10,000	← 33日 →	0
2.	1,000	← 〃 →	2,000	← 〃 →	10,000	← 〃 →	0
3.	1,000	← 〃 →	1,000	← 〃 →	10,000	← 〃 →	0
4.	1,000		—		10,000	← 〃 →	320
5.	—		—		10,000	← 〃 →	897

[Legend]: 1) [illegible] group; 2) First vaccination, number of X-ray (40,000 γ) irradiated Larvae; 3) Second vaccination, number of X-ray (40,000 γ) irradiated larvc; 4) Number of normal infected larvae; 5) Autopsy; number of vermes found in lung; 6) days.

In the group which received 1,000 to 4,000 larvae irradiated with 40,000 γ orally at 42 days intervals and even if 10,000 normally infected larvae were given twice orally, not a single vermes was found in the autopsy. However, in the group that was vaccinated only once, 820 vermes were found in the autopsy, and 897 vermes were found in the un-vaccinated group. In other words, it was proved that in the twice-vaccinated group all cattle acquired complete immunity. It was further clarified that the amount of the second vaccination, whether it was 1,000 or 4,000, did not make any difference in acquiring immunity. Although no great difference in the number of vermes detected existed between the once-vaccinated group and the un-vaccinated group, there was a remarkable difference in the growth of vermes. Thus, the vermes in the once-vaccinated group were not well developed; they were all immature vermes.

Jarrett, et al advanced various reasons why the acquisition of antigen immunity or by dead vermes was impossible, and why a far stronger immunity was acquired through X-ray irradiated larvae, compared with un-irradiated normal infected larvae. But, we believe that no convincing reasons have been found. Yet, Jarrett, et al explain that because the activity of X-ray irradiated larvae is partially hindered, when compared with normal infected larvae, it is impossible for the former to complete the normal migratory cycle in the body and mature. In their migratory cycle they are caught by the mesenteric lymphatic gland or the bronchomediastinal lymphatic gland, and sojourn there for a comparatively long period. Furthermore, Jarrett, et al explain that, because the complement fixation test is positive with the first vaccination of irradiated larvae, no change is observable in the antibody value of the complement fixation test after the second vaccination and further, after challenge infection, there may be no correlation between the antibodies of the complement fixation test and immunal antibodies. Consequently, as of now, there is no way of proving by in vitro testing the acquisition of immunity. In any case, that immunity may be acquired not by adult vermes but by infection of the larval stage is an interesting point. At present the irradiated larvae of cattle lung vermes are mass produced as "Dictol" in England and are being applied in practical use.

Furthermore, [illegible] et al (1959) clarified that by vaccination, irradiated larvae immunity could be acquired against Haemonchus contortus which parasites on the stomach of the sheep. Dow, et al (1959) clarified similarly on Uncinaria stenocephala, canine hookworm. These parasites do not take the complicated migratory course in the host as in the case of the lung vermes; instead they move directly from the mouth to the alimentary canal. It is interesting however, that even in this case immunity was acquired. It was also proved in the experiment on canine hookworm by Dow, et al that when the same number of normal infected larvae and irradiated larvae were given, a stronger immunity was acquired in the latter case by giving irradiated larvae.

On the Acquisition of Immunity Against Human Parasites

On immunity against schistosoma japonica:

The acquisition of immunity after curing schistosomiasis japonicum was pointed out previously by Dr. Fujinami. but Hsu, et al have conducted very interesting studies recently. Hsu, et al proved by human body experiments that schistosoma japonica in Taiwan would infect pigs rats, but not human beings, and that it had distinctly different biological characteristics from schistosoma japonica in Japan or in the Philippines. Yet because there are marked ecological differences between the two, schistosoma japonica in Taiwan is differentiated by calling it a non-human strain. Hsu, et al (1961) further infected monkeys with a non-human strain of schistosoma japonica, and discovered that the larvae infiltrated into the body migrated to the lung and other organs, but died as larvae without maturing. When these monkeys were infected with the human strain of schistosoma japonica, immunity was acquired. That is to say, with the non-human strain immunity was obtained. From these results, Hsu, et al further pointed out that the vermes in the larval stage played a large role in acquiring infection resistence to schistosomiasis japonicum.

It is already known that when the cercaria of different kinds of schistosoma of birds or rats, other than non-human schistosoma japonica penetrates the human skin, a strong dermatitis, so-called cercaria dermatitis, occurs. It is also known that when an intradermal test by the use of antigens manufactured from schistosoma japonica is made on human beings with cercaria dermatitis, they show marked positive reactions. Consequently, it is speculated that even through vaccinating with the cercaria of schistosoma of these animals resistence may be acquired.

Hsu, et al (1962) further proved, that as in the experiment by Jarrett, et al, animals vaccinated with X-ray irradiated cercaria acquired far stronger resistence than animals vaccinated with normal cercaria. They made several observations on the role of X-ray irradiation but these still contain unclear points. Other than schistosoma, similar experiments were made on trichinella spiralis, and in this case also, the acquisition of strong immunity was proven.

Conclusion

In the foregoing the author attempted to give an outline of the trends in recent immunological studies on parasites. An apology is due should difficulty arise because of the inadequacy of description. At any rate it is wonderful that the acquisition of immunity (infection resistence) to parasitic diseases is not impossible. It is hoped that this will succeed somehow in the future. We wish to make some contribution to the study in this field.

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